

Phytochemical Investigation and Anti-Inflammatory Evaluation of Isolated Compounds from the Stems of *Mimosa hamata* (Willd.)

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Abstract:

Background: Inflammation underlies the pathogenesis of a wide range of acute and chronic human diseases. Long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics is limited by gastric, renal and dependence-related adverse effects, prompting continued interest in plant-derived anti-inflammatory agents. *Mimosa hamata* (Willd.) (*Mimosaceae*) is used traditionally for urinary complaints, glandular swellings and as a general tonic, but its anti-inflammatory potential has not been previously characterized.

Objective: To evaluate the acute and chronic anti-inflammatory activity of a bioactive fraction (Fraction IV) and two isolated compounds (Compound I and Compound II) obtained from the ethanolic extract of the stems of *M. hamata* (ESMH).

Methods: Preliminary phytochemical screening of ESMH was performed. Acute anti-inflammatory activity was assessed using the carrageenan-induced rat paw oedema model, and chronic anti-inflammatory activity was assessed using the cotton pellet-induced granuloma model in Wistar albino rats. Aceclofenac (10 mg/kg) served as the reference standard. Paw volume and granuloma weight were compared across groups using one-way ANOVA followed by Dunnett's multiple comparison test.

Results: Preliminary phytochemical screening revealed the presence of Flavonoids, Carbohydrates, Glycosides And Tannins in ESMH. In the carrageenan model, Fraction IV (40 mg/kg) produced the greatest oedema inhibition among the test groups (84.09% at 5 h), followed by Compound I (64.77%) and Compound II (59.66%), compared with Aceclofenac (70.45%). In the cotton pellet granuloma model, Compound I produced the highest inhibition of granuloma dry weight (50.25%), followed by Compound II (45.94%) and ESMH (38.97%), compared with

Aceclofenac (57.33%).

Conclusion: The bioactive fraction and isolated compounds from the stems of *M. hamata* exhibit significant acute and chronic anti-inflammatory activity, plausibly mediated by their flavonoid content. These findings support further mechanistic and phytochemical characterisation of *M. hamata* as a source of anti-inflammatory lead compounds.

Keywords: *Mimosa hamata*; anti-inflammatory activity; carrageenan-induced paw oedema; cotton pellet granuloma; flavonoids

1. Introduction

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. Inflammation has been recognized as a major risk factor for various human diseases. The chronic inflammatory responses predispose to pathological progression of chronic illnesses, characterized by infiltration of inflammatory cells, excessive production of cytokines, dysregulation of cellular signalling and loss of barrier function; among these chronic illnesses rheumatoid arthritis is considered as the most common inflammatory disease and is a major cause of disability [2]. Attenuation of the chronic inflammatory response is a beneficial strategy to combat several human diseases. Although steroidal and non-steroidal anti-inflammatory drugs are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders because such compounds are accompanied by unexpected side effects [3,4]. Therefore, there is an urgent need to find safer anti-inflammatory compounds, and traditional medicine offers many plant extracts and pure natural compounds as treatment options of a wide variety of disorders including acute and chronic inflammation [5].

The use of medicinal parts is accepted as the most common form of traditional medicine. Among the entire flora, it is estimated that 35,000 to 70,000 species have been used for medicinal purpose. Some 5000 of these have been studied in biomedical research. In developing countries, herbal medicines continue to play important role in primary health care, especially where coverage of health service is limited. In industrialized countries, herbal medicine have become popular, However the expanded use of herbal medicine has lead to concerns relating to assurance of safety, quality and efficacy [6].

An acute interest in the phytochemistry and pharmacology of plant drugs is evident from the number of research papers published in different journals and through finding presented in national and international conferences. Furthermore, the facts that's the discovery of new synthetic drugs is time consuming expensive affair, and if we want to implement, "Health for all by the year 2000", then perhaps the logical choice would be in exploring plant products as potential medicines to maximum possible usage [7].

There is much hope for finding anti-inflammatory compounds from indigenous plants as these are still used in therapy despite the progress in conventional chemistry and pharmacology in producing the effective drugs.

Genus *Mimosa* (family: *Mimosaceae*) has about 400 species which are mainly shrubs & small trees in tropics. About 8 species are found in India, with medicinal importance [8] while some are of ornamental use. *Mimosa pudica* the curious plant in the genus is a creeping form. Because of the way it folds its leaves when touched, it is known as touch-me-not plant. *Mimosa hamata* also folds its leaves when touched [9]. *Mimosa hamata* is a much straggling shrub occurring in tropics & widely

distributed in India & Pakistan [10]. The plant is used for urinary complaints & as a tonic against general weakness. A paste of leaves is applied over glandular swellings & is used in dressing for sinus, sores & piles [11]. Its roots possess contraceptive efficacy while seeds are used as blood purifier [12]. Various bioefficacies viz., antifungal activity of deprotenized leaf extract [13,14]. Antibacterial activity of alcoholic extract of aerial parts, antiviral activity of methanolic extract of roots⁸ and Antioxidant activity [15] have been reported.

The major phytoconstituents present in *Mimosa hamata* (Willd.) include 4-ethylgallic acid from fresh flowers, triterpene saponin B (3-O-Larabinosyl-D-glucosyl morolic acid), mimonoside A, B, C and saponin A (3-O-D-glucosyl-L-rhamnosyl morolic acid) from the roots, ethylgallate and gallic acid from leaves [16].

The plant *Mimosa hamata* (Willd.) belonging to the family *Mimosaceae* is being selected for phytochemical investigations to pin point various pharmacological activities. The said plant has been reported to possess antibacterial, antiviral & antioxidant properties.[13,14,15] . It was found that no substantial work of the plant was carried out for its anti-inflammatory activity.

Materials and Methods:

Plant material:-

For the present study, stems of *M. hamata* plant were collected from Methwade, Tal. Sangola, Dist. Solapur (Maharashtra) and was authenticated at Botanical Survey of India, Pune. The voucher specimen will be deposited in the institution and in “Herbarium Department of Botany, Solapur University, Solapur” during the month of September 2015. The plant material was dried under shade at room temperature for about 15 days. The dried plant samples were powdered by mechanical grinder and sieved to give particle size 40-100 mm. The powder was stored in polythene bags at room temperature before extraction.

Preparation of Alcoholic extract:

The collected stems were shade dried, reduced to a coarse powder in a mechanical grinder to obtain of desired particle size (40# sieve). About 200 gm of powdered material was subjected to exhaustive extraction with 90% alcohol in a soxhlet extract or at a temperature of 60 –70⁰C, concentrated on a rotary flash evaporator at 50 ⁰C (Superfit, India), and finally to dry powder. Some part of the total extract was reserved for phytochemical investigation and rest of the extract was used for biological activity.

Preliminary Phytochemical analysis:

The ethanolic extract of stems of *M. hamata* was then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents, it revealed the presence of flavonoids, carbohydrate, glycosides, tannins. Preliminary Thin layer chromatography studies also confirmed these constituents[16].

Animals: Wistar albino rats weighing 175-225 g of either sex maintained under standard husbandary conditions (temp 23±2° C, relative humidity 55±10% and 12 hours light dark cycle) were used for the

screening. Animals were fed with standard laboratory food and water *ad libitum* during the study period. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, India 2009.

Toxicity studies: Acute toxicity study was performed for ethanolic extract according to the acute toxic classic method as per OECD guidelines 423[17,18].

Preparation of carrageenan suspension

Suspension of carrageenan sodium salt 1% was prepared by sprinkling 100mg of carrageenan powder in 10 ml of saline (0.9% NaCl) and set aside to soak for 1 hour, and then the suspension was mixed thoroughly using magnetic stirrer.

Preparation of sodium CMC suspension

Stock suspension of sodium CMC was prepared by triturating the powder sodium CMC (1g) finely in 2.5 ml of water, 1:10 dilution of this stock solution made in distilled water was used for suspending the test and standard drugs.

A) Evaluation of acute anti-inflammatory activity:

Carrageenan induced rat paw oedema model [19]

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly used techniques is based upon the ability of such agents to inhibit the oedema produced in the hind-paw of the rat after injection of phlogistic agent. Various measuring systems used for the assessment of induced oedema in the paw include: volume, paw thickness, paw weight and painfulness to monitor the development of the induced oedema in the paw. Acute hind paw oedema is induced either in mice or in rats by injecting 0.05mL to 0.1mL of 1%w/v carrageenan which reaches a peak oedema level at 2-3hrs after carrageenan injection. Although oedema can be induced by many other phlogistic agents like dextran, formaldehyde, 5- hydroxy-tryptamine, histamine, bradykinin, prostaglandin E₁ etc for routine screening, carrageenan induced oedema model is employed.

Anti-inflammatory activity was assessed using carrageenan induced rat paw oedema method. The ethical clearance was obtained before the experiment from the Institutional Animal Ethics Committee (Registration number 1406//PO/a/11/CPCSEA). Rats were kept in the polypropylene cages and fed on a standard laboratory diet with water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Rats were divided into 9 groups of six animals each.

Group-I: Rats were served as control received 0.1ml of 1% carrageenan (s.c.), 2.5ml of 0.5% w/v sodium carboxy methyl cellulose (C.M.C); p.o.

Group-II: Rats were received Aceclofenac sodium (10mg/kg/p.o.)

Group-III: Rats were received 1.0ml of ethanolic extract of stems of *Mimosa hamata* (400mg/kg/o.p)

Group-IV: Rats were received 1.0ml of Fraction-IV of ethanolic extract of stems of *Mimosa hamata* (400mg/kg/o.p)

Group-V: Rats were received 1.0ml of isolated Compound-I (20mg/kg/o.p) from fraction-IV of ethanolic extract of stems (400mg/kg) of *Mimosa hamata*

Group-VI: Rats were received 1.0ml of isolated Compound-II (20mg/kg/o.p) from fraction IV of ethanolic extract of stems (400mg/kg) of *Mimosa hamata*

One hour later, the rats of group III, IV, V, VI were challenged by subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan into the planter side of the left hind-paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection (0 h) and followed by every hour till the 6th hr after injection of carrageenan to each group. The paw volume up to the tri-biotal articulation was measured at 0, 1, 2, 3, 4 and 5th hours. The difference between the initial and subsequent reading gave the actual oedema volume.

Percent inhibition of inflammation was calculated using the formula,

$$\% \text{ Edema Inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

where 'V_c' represents oedema volume in control and 'V_t', oedema volume in group treated with test extracts. The values obtained from each group were expressed as Mean $\pm$ Standard deviation. Dunnet's t- test was done to compare the statistical significant changes between control, Carrageenan induced paw odema, Aceclofenac treatment rats and with extract treatment. The significant levels between the groups were compared using row wise comparison between Initial with different hours.

Effect of oral administration of isolated compounds of ethanolic extract of stems from *Mimosa hamata* (Willd.) in carrageenan induced paw edema in rats

The isolated compounds are active component and produce potent therapeutic activity compared to crude extract. Hence, one tenth part of extract dose (400 mg/kg) was selected for the fraction IV isolated from the ethanolic extracts of stems (400mg/kg) of *Mimosa hamata*. Further one tenth part of ethanolic extracts of isolated compound (40mg/kg) was selected to evaluate anti-inflammatory activity of fraction IV. Still further, one half of the isolated compounds i.e. 20 mg /kg was selected for both the isolated

Table no.1: Effects of the Isolated Compounds from ethanolic extracts of stems of *Mimosa hamata* (Willd.) on the Carrageenan-Induced Rat Paw Oedema Model

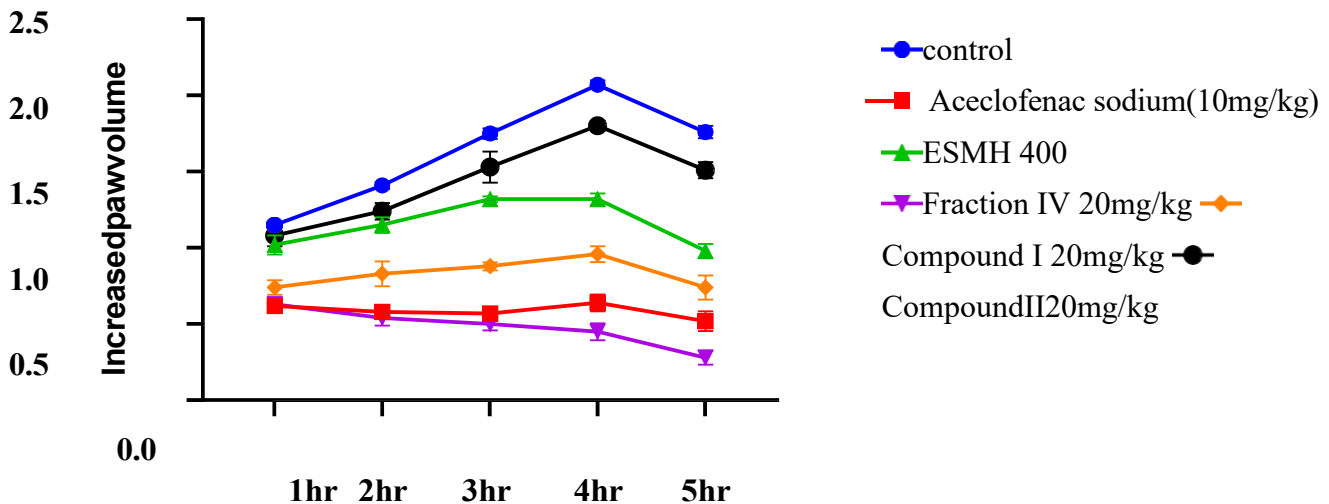
Treatment& Dose(mg/kg, b.w.)	Paw edema volume Mean $\pm$ SEM				
	1h	2h	3h	4h	5h
Control	0.24 $\pm$ 0.01	0.26 $\pm$ 0.01	0.24 $\pm$ 0.01	0.24 $\pm$ 0.01	0.25 $\pm$ 0.01

Carrageenan 1% w/v	1.15±0.038#	1.41±0.033#	1.75±0.035#	2.07±0.028#	1.76±0.040#
Aceclofenac (10mg/kg b.w.)	0.62±0.023* (46.08)	0.58±0.040* (58.86)	0.57±0.040* (67.42)	0.64±0.055* (69.08)	0.52±0.064* (70.45)
ESMH 400mg/kg	1.02±0.063 (11.30)	1.15±0.051 (18.43)	1.32±0.018* (24.57)	1.32±0.038* (36.23)	0.98±0.046* (44.32)
Fraction IV (40 mg/kg) ESMH	0.63±0.018* (45.21)	0.54±0.049* (61.70)	0.50±0.041* (71.43)	0.45±0.056* (78.26)	0.28±0.046* (84.09)
Comp-I (20 mg/kg b.w.)	0.74±0.048* (35.65)	0.83±0.081* (41.13)	0.88±0.026* (49.71)	0.96±0.052* (53.62)	0.62±0.079* (64.77)
Comp-II (20 mg/kg b.w.)	1.08±0.068 (6.15)	1.24±0.054 (12.05)	1.53±0.102 (12.57)	1.80±0.032 (13.04)	0.71±0.053 (59.66)

Figures in bracket indicate oedema inhibition percentage.

Values are expressed as Mean±SEM, (n=6). *p<0.05, **p<0.01, ***p<0.001, using one way ANOVA coupled with Dunnett's multiple comparison test by Graph-pad prism software 8.4.3, criterion for significance ***p<0.001 is considered as significant. # indicates control group compared with normal and * indicates all others groups compared with control group.

Carrageenan-Induced Rat Paw Oedema



Time in hr

Figure No1:- Changes in rat paw volume in carrageenan induced paw edema

Carrageenan-Induced Rat Paw Oedema

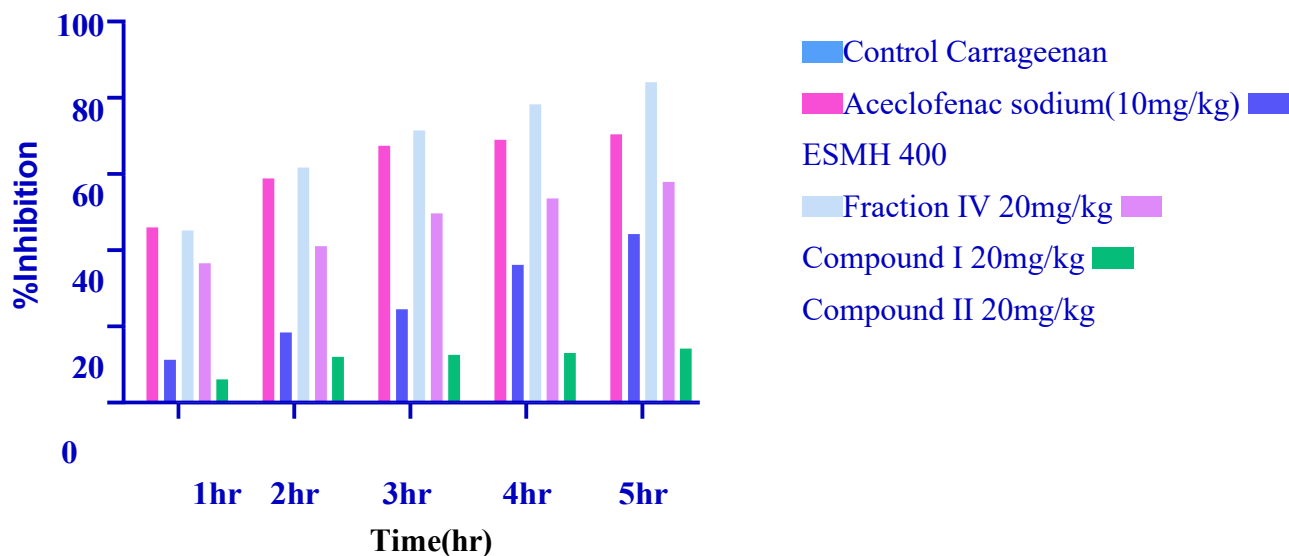


Figure No. 2: Percent inhibition of rat paw volume in carrageenan induced paw edema

Table no. 2: Percent Inhibition of rat paw volume in carrageenan induced paw edema

Groups	Initial paw volume	5 hr	Difference in Paw	Inhibition percentage
Control	0.24±0.01	0.25±0.01	0.01	-
Carrageenan(1%w/v)	1.15±0.038	1.76±0.040	-0.61	-
Aceclofenac(10mg)	0.62±0.023* (46.08)	0.52±0.064* (70.45)	0.10	49.12%
ESMH(400mg/kg)	1.02±0.063 (11.30)	0.98±0.046* (44.32)	0.04	37.5%
Fraction-IV(40mg/kg)	0.63±0.018* (45.21)	0.28±0.046* (84.09)	0.35	39.34%
Compound-I (20mg/kg)	0.74±0.048* (35.65)	0.62±0.079* (64.77)	0.12	22.38%
Compound-II (20 mg/kg)	1.08±0.068 (6.15)	0.71±0.053 (59.66)	0.37	24.61%

B) Evaluation of chronic anti-inflammatory activity:**Cotton pellet induced granuloma:**

The method of Winter and Porter was used to study chronic inflammation in rats.

Albino Wistar rats were randomly divided into eight groups of six animals in each group were taken, anaesthetized with ether. The axillary skin was shaved and disinfected with 70% ethanol. An incision was made and by a blunt forceps subcutaneous tunnels were formed and a sterilized cotton pellet (50±1mg) was placed in both axillas. All the animals in each group were treated in the following way

Group-1: Served as Toxicant control, which received orally 1 ml/kg of 1% sodium CMC solution daily for 8 days following subcutaneous implantation of cotton pellets.

Group-2: Termed as Standard and received Aceclofenac sodium 10mg/kg p.o. once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-3: Received ethanolic extracts of stems of *Mimosa hamata* 400mg/kg once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-4: Received received 1.0ml of Fraction-IV of ethanolic extract of stems of *Mimosa hamata* (400mg/kg/o.p) once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-5: Received received 1.0ml of isolated Compound- I (20 mg/kg/o.p) from fraction IV of ethanolic extract of stems (400mg/kg) of *Mimosa hamata* once daily orally for 8 days following subcutaneous implantation of cotton pellets.

Group-6: Received received 1.0ml of isolated Compound-II (20mg/kg/o.p) from fraction IV of ethanolic extract of stems (400mg/kg) of *Mimosa hamata* once daily orally for 8 days following subcutaneous implantation of cotton pellets.

On the 9th day, the animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture. The cotton pellets along with granuloma tissue were removed and weighed immediately for wet weight. The pellets were dried in an oven at 60°C until a constant weight was obtained.

The granuloma tissue formation and exudate formation was calculated using the following formulae:

Measure of granuloma tissue formation = constant dry weight – initial weight of pellet

The level of inhibition of granuloma tissue development was calculated using the expression:

Tableno.3:-Cotton pellet granuloma

Treatment	Dose	Weight of dry cotton pellet	%Inhibition
Control	Vehicle	84.66±4.33	00
Aceclofenac	10 mg/kg	36.12±1.84*	57.33
ESMH	400 mg/kg	51.66±3.11*	38.97
Fraction-IV	40 mg/kg	47.44±2.11*	43.96
Compound I	20 mg/kg	42.11±1.98*	50.25
Compound II	20 mg/kg	45.76±1.98	45.94

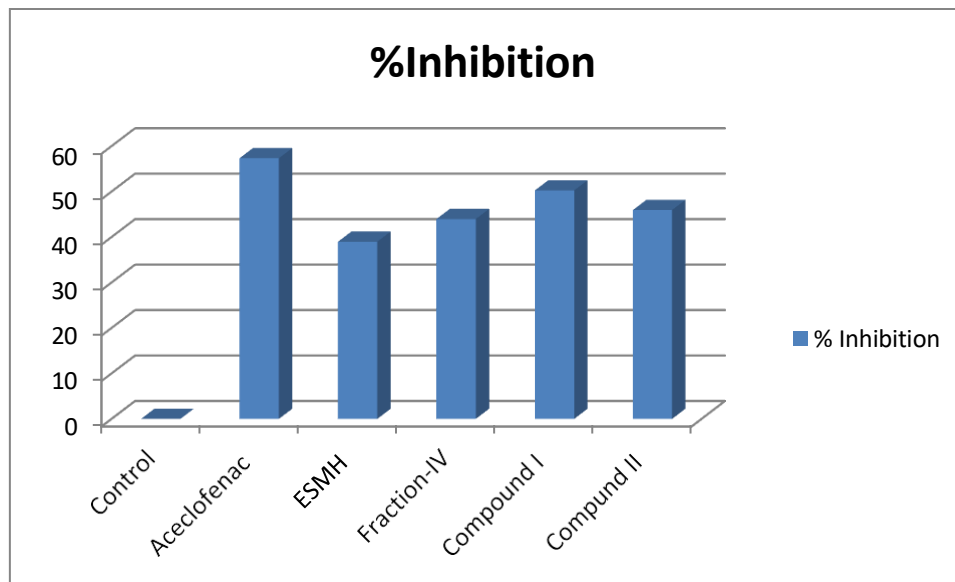


Figure no. 2: Percent inhibition of cotton pellet granuloma

Statistical Analysis:-

All the results were expressed as mean $\pm$ SD. Analyses were processed using Graph Pad Prism software for Windows (version 8.4.6, Graph Pad Software). The significance of difference among the studied groups was determined using one-way analysis of variance (ANOVA). Values are expressed as mean $\pm$ SEM, (n=6). *p<0.05, **p<0.01, ***p<0.001, using one-way ANOVA coupled with "Dunnnett" multiple comparison test by Graph-pad prism software 8.4.3, criterion for significance ***p<0.001 is considered as significant. # indicates control group compared with normal and * indicates all others groups compared with control group.

Results:-

Preliminary phytochemical screening of ethanolic extract of the stems of *Mimosa hamata* (Willd.) revealed the presence of bioactive components like flavanoids, carbohydrate, glycosides and tannins.

Carrageenan induced rat paw edema

A response associated with the cardinal signs of redness, heat, pain and swelling is inflammation. The body adopts this mechanism to enclose the inflammation when a tissue is injured. It subsides within a few days. The standard experimental model of acute inflammation is carrageenan - induced hind paw edema. For several biological activities, *Mimosa hamata* stems were extensively studied. For medicinal purposes in Ayurveda, Siddha and Unani, the aerial part of the plant is used.

Carrageenan induced edema is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and kinins and then prostaglandin in the later phase [20]. So, the effect of the ethanolic extract against inflammations produced by these individual mediators was studied.

Flavonoids and other phenolics compounds of plant origin have been reported as antioxidants and as scavengers of free radicals [21,22,23]. Considering that antioxidants can also exert anti-inflammatory effects [24]. The extract effectively suppressed the inflammation produced by histamine, bradykinin, prostaglandins and serotonin Table 2.

Carrageenan induced paw edema model was used for the evaluation of anti-inflammatory activity of the ethanolic extract of stems *M. hamata*. There was a dose-dependent, significant reduction in carrageenan-induced rat paw edema at 400 mg/kg of ethanolic extracts of *M. hamata* and at 10 mg/kg Aceclofenac over a period of 5 hrs as shown in Table 1 & 2.

In the carrageenan induced acute inflammation, Group 3 treated rats (i.e. standard drug Aceclofenac in a dose of 10 mg/kg, b.w., p.o., treated rats) showed significant activity (p<0.001) at 1st and 2nd hour with respective percentage inhibitions of 46.08% and 58.86% with respect to Group-1 rats (i.e. controlled rats). It showed highly significant (p< 0.001) activity starting from 3rd and lasting up to 6th hour. The percentage inhibitions were observed to be 67.42%, 69.08%, 70.45% respectively

Group 4 rats (i.e. ethanolic extract of stems of *Mimosa hamata* 400 mg per kg b.w., p.o.) showed significant activity from 1st and 2nd hour with respect to percentage inhibition of 11.30% and 18.43% when compared to Group-1 rats (i.e. controlled rats). The extract appears to show highly significant

activity from 3rd to 5th with percentage and division of extract 24.57%, 36.23%, 44.32%

Group-5 rats (i.e. Fraction IV from ethanolic extracts of stems of *Mimosa hamata* in a dose of 40 mg/kg b.o. p.o) showed significant anti-inflammatory activity at 1st and 2nd hour with percentage inhibition of 45.21% and 61.70% when compared to group -1 rats (i.e. controlled rats). The extract appeared to show highly significant activity from 3rd to 5th hr with respect to 71.43%, 78.26%, 84.09% when compared to group-1 rats (i.e. controlled rats)

Group- 6 rats (i.e. isolated compound I from Fraction IV of ethanolic extract of stems of *Mimosa hamata* 20 mg/kg b.w. p.o.) appears to show significant anti-inflammatory activity at 1st and 2nd hour with percentage inhibition of 35.65% and 41.13 % respectively. It appears to show a highly significant (p<0.001) anti-inflammatory activity from 3rd to 5th hour with respect to percentage inhibition of 49.71%, 53.62%, 64.77% when compared to group -1 rats (i.e controlled rats)

Group- 7 rats (i.e isolated Compound II from Fraction IV of ethanolic extract of stems of *Mimosa hamata* 20 mg/kg b.w.p.o.) appears to show significant anti-inflammatory activity at 1st and 2nd hour with percentage inhibition of 6.15% and 12.05 % respectively. It appears to show a highly significant (p<0.001) anti-inflammatory activity from 3rd to 5th hour with respect to percentage inhibition of 12.57%, 13.04%, 59.66% when compared to group -1 rats (i.e controlled rats)

Cotton Pellet Induced Granuloma

The cotton pellet method is extensively used to evaluate the transudative and proliferative components of the chronic inflammation. In cotton pellet induced granuloma model, granuloma develops within several days and this model of inflammation is used to study the proliferative phases of inflammation. Granuloma is formed due to the proliferation of macrophages, neutrophils and fibroblasts which are the basic sources of granuloma formation.

Chronic inflammatory condition was induced in animals by inserting the cotton pellets subcutaneously. The results of cotton pellet granuloma method revealed that compounds I & II isolated from fraction IV of ethanolic extract of stems 400mg/kg of *Mimosa hamata* (Willd.) was found to decrease the weight of granuloma significantly (50.25% & 45.94%) than the ethanolic extract of stems of *M. hamata* at 400mg/kg (38.97%)

Discussion:-

The results of this study indicate that the stems extracts of *Mimosa hamata* possess acute and chronic anti-inflammatory activity against various phlogistic agents. Inflammation induced by carrageenan involves three distinct phases of the release of the mediator, including serotonin and histamine in the first phase (0 – 2 h), kinins in the second phase (3 h), and prostaglandin in the third phase^[4] (>4 h). The fraction IV ethanolic extract of stems of *Mimosa hamata* 400mg/kg and isolated compounds I & II from fraction IV of ESMH 400 mg/kg significantly inhibited paw edema induced by carrageenan in the first phase by Fraction IV of ESMH suggesting an inhibitory effect on the release of histamine and/or serotonin and in the second & third phase of isolated compounds I & II, indicating the possible inhibition of mediator of acute inflammation in this phase i.e. prostaglandin.

Conclusion:-

The outcomes from the present study displayed that the isolated compounds from the fraction IV of <https://doi.org/10.5281/zenodo.21483961> || Page No - 47

ethanolic extracts of *Mimosa hamata* comprised of flavonoids, and prominently inclined their antioxidant properties. The isolated compounds from the fraction IV of ethanolic extracts of *M. hamata* 400 mg/kg demonstrated significant anti-inflammatory activity, and suggesting that flavonoids is the main contributor for anti-inflammatory activity. Further studies are carried for the possible mechanism and the characterization of the bioactive component responsible for anti-inflammatory activity.

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